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Association between Uteroferrin, Retinol-Binding Protein, and Transferrin within the Uterine and Conceptus Compartments during Pregnancy in Swine[1]

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ABSTRACT

The association between uteroferrin (UF, measured as acid phosphatase [AP] activity), retinol-binding protein (RBP), and transferrin (TF) within the intrauterine environment during the estrous cycle and pregnancy was examined. Pregnant gilts were killed on Days 7, 10, 13, 16, 19, 25, 30, 40, 50, 60, 70, 80, and 90. Cyclic gilts were killed on Days 7, 10, 13, and 16. On Days 7 through 19, each uterine horn was flushed with 20 ml Minimum Essential Medium. On Days 25 through 90, serum and allantoic fluid samples were collected from each fetus within each gilt and pooled for each gilt. Uterine flushings, allantoic fluid, and fetal serum samples were assayed for AP, RBP, and TF. Endometrium was collected from all gilts and cultured with [^{3}H]-leucine; conditioned medium was measured for nondialyzable radioactive macromolecules, AP, and RBP. In uterine flushings, AP, RBP, and TF were low on Day 7, and then increased markedly ($p < 0.01$) by Day 13 in both cyclic and pregnant gilts. After log transformation of the data, AP was highly correlated with RBP ($r = 0.99$) and TF ($r = 0.91$, $p < 0.01$). Secretion of RBP and AP by endometrium in culture also increased ($p < 0.01$) during this period and was similar in cyclic and pregnant gilts, and RBP and AP secretion were highly correlated ($r = 0.65$, $p < 0.01$). Endometrial tissue did not secrete detectable amounts of TF in culture. Concentrations of all three proteins were low in allantoic fluid on Day 25, after which time they increased to Day 40, and then either stabilized to Day 70 followed by a decrease to Day 90 (AP and TF) or decreased to Day 90 (RBP). Endometrial secretion of both AP and RBP increased between Day 25 and 30. Then, AP increased further to Day 40 and did not change to Day 60, while RBP did not change from Day 30 to Day 60. Both decreased from Day 60 to 70, did not change to Day 80, and then increased again to Day 90. The association of RBP and TF concentrations with AP in various maternal and conceptus compartments during pregnancy is consistent with the hypothesis that one function of these two proteins may be to protect maternal and fetal tissues from lipid peroxidation that is a possible consequence of iron transport via endometrial secretion of UF.

INTRODUCTION

During pregnancy in pigs, the endometrium secretes several proteins [1]. Many of these proteins are known or hypothesized to transfer nutrients to the developing conceptus. Two such proteins are uteroferrin (UF), which transfers iron [2], and retinol-binding protein (RBP) [3], which transfers retinol. A great deal of information has accumulated on the pattern of secretion of UF during both the estrous cycle and pregnancy. It has been shown that at the time of maternal recognition of pregnancy, a large increase in uterine secretion of UF occurs [4, 5]. A second large increase in UF secretion occurs after Day 30 of pregnancy, and the peak secretion rate of this protein by the endometrium occurs around Day 60 of pregnancy [6].

It has recently been demonstrated that UF, like other iron-containing proteins excluding transferrin (TF), is capable of catalyzing lipid peroxidation [7] in the presence of concentrations of ascorbic acid that are similar to those likely to be present within the uterine lumen [8] and fetal fluids [9]. Because uncontrolled lipid peroxidation is known to result in cell damage or death, the intrauterine environment probably has mechanisms to prevent this reaction. Both RBP and TF inhibit this reaction in vitro [7]. If these two proteins protect tissues from damage caused by UF in vivo, it seems likely that secretion and/or concentrations of these proteins in the various intrauterine compartments would be correlated with UF concentrations.

Endometrial concentrations of mRNA for RBP throughout the estrous cycle and pregnancy have been reported previously [10–12]. Relative concentrations of mRNA for RBP increase at the time of maternal recognition of pregnancy as does the intrauterine content of retinol [10, 11]. Peaks in endometrial RBP mRNA concentrations occur at Day 30 and at term [12]. However, actual concentrations of RBP within the intrauterine environment or in fetal fluids have not been examined, nor has the relationship between RBP and UF concentrations within the intrauterine environment been specifically studied.

The concentration of TF within the various intrauterine and conceptus compartments has not been extensively studied. Fetal serum TF concentrations are low on Day 23 of pregnancy, increase by Day 37 of pregnancy, and then fall again after Day 70 of pregnancy [13, 14]. Transferrin content within the uterine lumen during early pregnancy and in allantoic fluid has not been reported. However, a protein similar to TF is secreted by the porcine conceptus during early pregnancy [15], and TF is known to be present in allantoic fluid [9]. The association between UF and TF concentrations within the intrauterine environment throughout the estrous cycle and pregnancy has also not been investigated.

The objectives of this study were 1) to determine the association between UF, TF, and RBP within the intrauterine environment during the estrous cycle and early pregnancy and within allantoic fluid and fetal serum during later pregnancy and 2) to characterize secretion of UF, RBP, and TF by the endometrium during the estrous cycle and pregnancy.

MATERIALS AND METHODS

White crossbred pigs (10–12 mo of age) were checked daily for estrus through two to three estrous cycles before being assigned to treatments. On the first day of standing estrus (Day 0), gilts were assigned randomly to remain cyclic or to be mated. Cyclic gilts were slaughtered on Days 7, 10, 13, or 16 (n = 4 for Day 13 and 5 for all other days)

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of the estrous cycle. Pregnant gilts were slaughtered on Days 7, 10, 13, 16, 19, 25, 30, 40, 50, 60, 70, 80, or 90 (n = 6 for Day 10; 5 for Days 7, 13, 16, 19, and 30; and 3 for all others) of pregnancy. For gilts slaughtered on Day 19 or earlier, each uterine horn was flushed with 20 ml Minimum Essential Medium containing one tenth the normal amount of leucine (MEM). Endometrial tissue was collected near the middle of each uterine horn and placed into 20 ml MEM. For gilts slaughtered on Day 25 or later, 5 ml allantoic fluid was collected through the uterine wall from each conceptus within a gilt and pooled for each gilt. The remainder of the allantoic fluid was collected after rupture of the allantoic sac, and the volume was measured for each fetus. For gilts slaughtered on Day 30 or later, fetal blood (up to 1 ml) was collected from the umbilical cord of each fetus within a gilt. These samples were pooled and allowed to clot; serum was then collected by centrifugation (1000 × *g*). Placentae from two conceptuses near the middle of each uterine horn were dissected from the endometrium, and endometrial tissue was then collected into 20 ml MEM. Endometrial tissues (500 mg) from all gilts were cultured in the presence of 50 μCi [^{3}H]-leucine in serum-free MEM using conditions described previously [16].

Protein was measured in uterine flushings and allantoic fluid using the method of Lowry et al. [17] with BSA as standard. To eliminate the effect of phenol red in samples of uterine flushings, protein was precipitated from 300 μl of uterine flushing samples by the addition of 900 μl of 20% trichloroacetic acid followed by centrifugation (13 000 × *g*). Protein pellets were redissolved in 1 M NaOH and assayed for protein. Protein secretion into the endometrial culture medium was measured as nondialyzable radioactive macromolecules (NRM). Culture medium was dialyzed (molecular weight cutoff = 3500) at 4°C against three overnight changes of 8 L of 10 mM Tris, pH 8.2; then 100 μl was subjected to scintillation counting.

Retinol-binding protein in uterine flushings, allantoic fluid, fetal serum, and endometrial culture medium was measured by RIA [18]. Intra- and interassay coefficients of variation for these assays were 12.9% and 8.1%, respectively.

Relative UF concentrations in uterine flushings, allantoic fluid, fetal serum, and endometrial culture medium were measured as acid phosphatase (AP) activity [4–6] using the procedure described by Vallet and Christenson [19].

Transferrin in uterine flushings, allantoic fluid, and fetal serum was measured using ^{59}Fe blotting after electrophoresis of samples by a modification of the procedure of Makey and Seal [20]. Briefly, samples were lyophilized and redissolved in 0.375 M Tris (pH 8.2) and 6 M urea. The running gel was 5% acrylamide containing 0.375 M Tris (pH 8.2) and 6 M urea. Chamber buffer was 25 mM Tris and 192 mM glycine. Samples were electrophoresed for 1 h at 20 mA per gel and then overnight at 10 mA. After electrophoresis, gels were soaked in fresh chamber buffer for 10 min and then electroblotted onto nylon-supported nitrocellulose for 4 h at 4°C and 0.5 amps. Blots were stored when necessary in 10 mM Tris, pH 8.2. For ^{59}Fe ligand blotting, blots were soaked in four changes of 100 mM sodium acetate, 100 mM sodium citrate, pH 4.5, to remove endogenous-bound iron (TF retains its iron in 6 M urea). Blots were rinsed in 100 mM Tris, 1 mM sodium citrate, 10 mM sodium bicarbonate (pH 8.0) and then incubated for 2 h at room temperature with ^{59}Fe in the Tris-citrate-bicarbonate buffer (0.5 μCi/ml, 10 ml per blot). Blots were rinsed four times in Tris-citrate-bicarbonate buffer and then subjected to autoradiography while still wet. After autoradiography, blots were allowed to air dry, and TF-bound iron was located on the blots using the autoradiographs as a guide. Sections of the blot containing ^{59}Fe bound to TF were cut from each sample lane and subjected to scintillation counting to quantify the amount of ^{59}Fe bound. A similar-sized area of the blot that did not contain ^{59}Fe bound to TF was used to determine nonspecific ^{59}Fe binding, and this was subtracted from all sample counts. To standardize each set of blots, a sample of Day 60 allantoic fluid from a large pool was run in one lane of each blot, an average was calculated for the ^{59}Fe bound by these samples for all the blots in each set, and all results were expressed as relative units corresponding to the average ^{59}Fe bound by this sample for blots in the set (i.e., one unit = the amount of TF in 1 ml of the Day 60 allantoic fluid pool). Uteroferrin is not detected using this procedure because of its basic isoelectric point. To validate that the ligand blot assay accurately reflected relative TF concentrations in samples, ^{59}Fe bound by increasing volumes of allantoic fluid and fetal serum was compared to increasing amounts of purified porcine fetal TF. Fetal TF was purified from Day 60 allantoic fluid using the procedure described by Buhi et al. [9]. SDS-gel electrophoresis of 10 μg purified TF resulted in a single approximately 80 000 M_r band after Coomassie staining (not shown). To determine whether endometrial tissues secrete TF in culture, three 1-ml aliquots of dialyzed culture medium from incubations of endometrium from three Day 16 pregnant and three Day 16 cyclic gilts were lyophilized and subjected to two-dimensional (2D)-PAGE [21]. One 2D-PAGE gel for each gilt was subjected to fluorography using procedures described by Roberts et al. [21]. The other two gels were blotted onto nylon-supported nitrocellulose and immunostained using goat anti-human transferrin (Sigma Chemical Co., St. Louis, MO) or normal goat serum (1:2000 dilution) followed by anti-goat IgG-peroxidase (Sigma) and diaminobenzidine as the color indicator according to procedures described previously [22]. This allowed immunolocalization of TF migration during 2D-PAGE and comparison of this migration with the migration of potentially radioactive proteins secreted by the endometrium.

Statistical Analysis

Protein, AP, RBP, and TF content in uterine flushings collected during the estrous cycle or early pregnancy were calculated by multiplying concentrations by the volume of flushing obtained for each gilt and were examined by analysis of variance using the effect of status (cyclic or pregnant), day, and status by day as the model. Concentrations and content of protein, AP, RBP, and TF in allantoic fluid and concentrations of AP, RBP, and TF in fetal serum collected throughout pregnancy were examined by analysis of variance using the effect of day as the model. Average endometrial secretion of NRM, RBP, and AP in culture was calculated for each gilt, and these data were analyzed using the effect of status, day, and status by day interaction (Days 7–19) or the effect of day (Days 25–90). Because of unequal variances between days, all data were log transformed before analysis. Contrasts were used to further examine differences between treatment means. Simple correlations between all the factors measured were also calculated using log-transformed data. Correlations between AP, RBP, and TF in uterine flushings were also calculated after fitting protein in uterine flushings as a covariate.

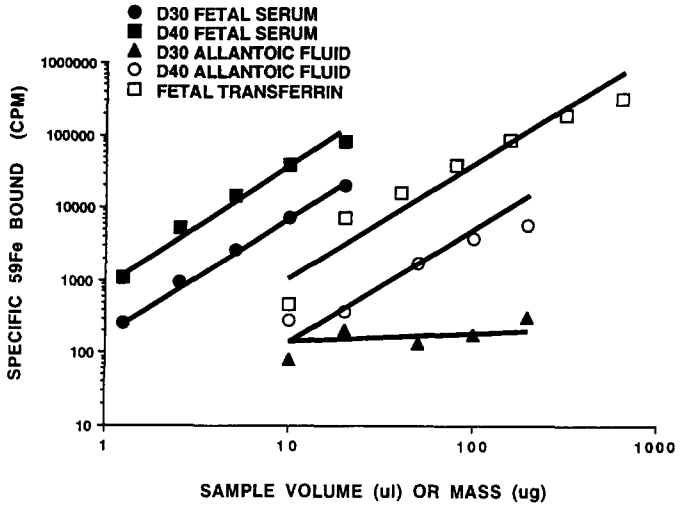

FIG. 1. The effect of increasing sample volume (fetal fluids) or mass (purified TF) on specific ^{59}Fe bound using ^{59}Fe ligand blotting. Transferrin in the Day 30 allantoic fluid sample examined was below detection at the volumes used; all other fluids were parallel with purified TF using homogeneity of regression analysis.

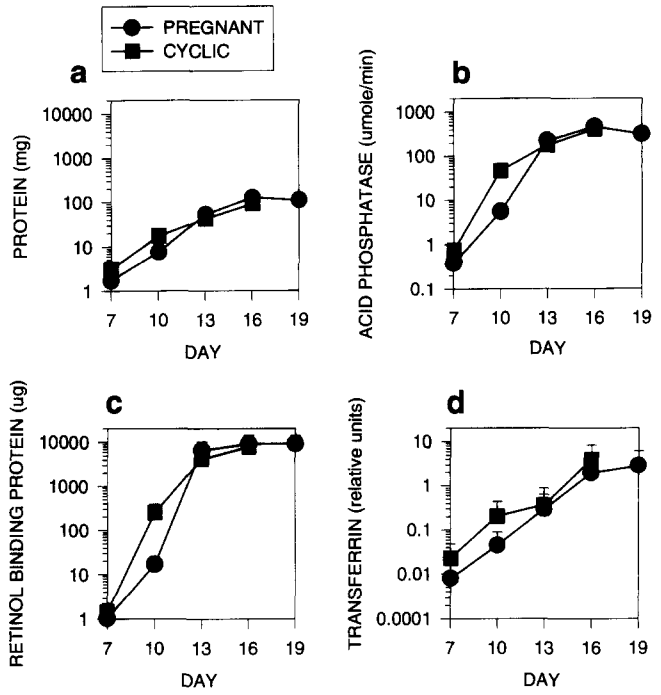

FIG. 2. Least-squares means (± SEM) for protein (**a**), AP (**b**), RBP (**c**), and TF (**d**) in uterine flushings from early cyclic and pregnant gilts are illustrated. All four increased ($p < 0.01$) from Day 7 to Day 13 or 16 and were strongly correlated (see Table 1). Note the log scales.

RESULTS

The amounts of ^{59}Fe bound by increasing volumes of allantoic fluid and fetal serum were parallel with the amount of ^{59}Fe bound by increasing amounts of purified porcine fetal TF (Fig. 1). The coefficient of variation of ^{59}Fe bound by the Day 60 allantoic fluid pool between blots within a blotting run averaged 7.8%. The coefficient of variation between the two blotting runs was 9.2%. These results indicate that ligand blotting is capable of measuring relative TF concentrations in samples.

Least-squares means for protein, AP, RBP, and TF content in uterine flushings from cyclic and pregnant gilts up to Day 19 are illustrated in Figure 2. A moderate increase ($p < 0.01$) in all four factors occurred between Days 7 and 10 with a large increase occurring between Days 10 and 13. A further increase occurred in TF from Day 13 to Day 16. Intrauterine content of protein, AP, and RBP did not change from Day 13 to Day 19. A status by day interaction was detected ($p < 0.05$) for protein, AP, and RBP. This was primarily due to increased protein ($p < 0.05$), AP ($p < 0.01$), and RBP ($p < 0.01$) on Day 10 in cyclic gilts compared to pregnant gilts. Protein, AP, RBP, and TF content in uterine flushings did not differ between cyclic and pregnant gilts on Days 13 or 16, and these four factors were strongly correlated with each other (Table 1) with the greatest correlation occurring between AP and RBP. After fitting protein as covariate, RBP remained highly correlated with AP ($r = 0.88$; $p < 0.01$).

Secretion of NRM, AP, and RBP by endometrium did not differ between cyclic and pregnant gilts and followed a pattern similar to that obtained for uterine flushings (Fig. 3). Secretion of NRM ($p < 0.05$) and RBP ($p < 0.01$) increased from Day 7 to 13 and did not change from Day 13 to 19. Secretion of AP into culture medium increased from Day 7 to 10 ($p = 0.05$) and did not change from Day 10 to 19. Immunoblotting demonstrated the presence of large amounts of TF in endometrial culture medium, probably resulting from serum contamination (Fig. 4). However, a corresponding radioactive protein was not detected using fluorography, suggesting that the endometrium does not synthesize and secrete detectable amounts of TF.

Allantoic fluid concentrations (Fig. 5a) and content (Fig. 5b) of protein, AP, and RBP increased from Day 25 to 30 ($p < 0.01$) and from Day 30 to Day 40 ($p < 0.01$) of pregnancy. Allantoic fluid concentrations of TF were not different from Day 25 to 30 while TF content increased ($p = 0.01$), and both concentration and content increased ($p < 0.01$) from Day 30 to 40 of pregnancy. Allantoic fluid concentrations of protein, AP, and TF were not different from Day 40 to Day 80 of pregnancy; RBP concentration decreased ($p < 0.05$) from Day 40 to 50 and was then not different to Day 80. Protein concentration remained not different from Day 80 to Day 90 while AP ($p < 0.05$), RBP ($p < 0.01$), and TF ($p < 0.01$) concentrations decreased by Day 90. Partly due to changes in allantoic fluid volume, allantoic fluid protein content increased from Day 40 to 50 ($p < 0.05$), increased from Day 50 to 60 ($p < 0.05$), decreased from Day 60 to 70, and then was not different to Day 90. Allantoic fluid AP content was not different from Day 40 to 50, increased from Day 50 to 60 ($p < 0.01$), was then not different to Day 80, and thereafter decreased ($p < 0.05$) to Day 90. Allantoic fluid RBP content was not different and TF content increased ($p < 0.01$) from Day 40 to 50; then allantoic fluid content of both proteins was not different from Day 50 to 60, decreased from Day 60 to 70, and thereafter was not different to Day 90.

Endometrial secretion of NRM, AP, and RBP increased from Day 25 to 30 (Fig. 6). Endometrial secretion of NRM was not different from Day 30 to Day 70, increased ($p < 0.05$) from Day 70 to Day 80, and was not different from Day 80 to Day 90. Endometrial secretion of AP increased ($p < 0.01$) further from Day 30 to Day 40, while RBP secretion was not different. Both AP and RBP secretion were not different from Day 40 to 60, decreased ($p < 0.05$) from Day 60 to 70, were not different from Day 70 to 80, and then increased ($p < 0.01$) from Day 80 to 90.

Concentrations of AP, RBP, and TF in fetal serum are

TABLE 1. Correlations between protein (P), AP, RBP, TF, and nondialyzable radioactivity (NRM) in uterine flushings (UT; content), secreted by endometrial culture (EC; μg/g tissue), in allantoic fluid (AF; concentrations), or in fetal serum (FS; concentration) during either the cycle/early pregnancy (n = 45) or later pregnancy (n = 25).

	UT-AP	UT-RBP	UT-TF	AF-AP	AF-RBP	AF-TF	EC-NRM	EC-AP	EC-RBP	FS-AP	FS-RBP	FS-TF
UT-P[c]	.96[b]	.95[b]	.90[b]				.67[b]	.54[b]	.88[b]			
UT-AP		.99[b]	.91[b]				.76[b]	.64[b]	.94[b]			
UT-RBP			.90[b]				.76[b]	.63[b]	.95[b]			
UT-TF							.64[b]	.59[b]	.84[b]			
ECNRM								.68[b]	.80[b]			
ECAP									.65[b]			
AF-P				.97[b]	.62[b]	.94[b]	.61[b]	.73[b]	0	.03	.64[b]	.12
AF-AP					.68[b]	.93[b]	.65[b]	.75[b]	.05	.19	.59[b]	.23
AF-RBP						.68[b]	.21	.36	.03	.81[b]	.19	.65[b]
AF-TF							.53	.67[b]	−.15	.27	.49[a]	.39
EC-NRM								.88[b]	.32	−.64[b]	.17	−.21
EC-AP									.44[a]	−.57[a]	.34	−.04
EC-RBP										−.25	−.31	−.24
FS-AP											−.13	.72[b]
FS-RBP												.09

[a] $p < 0.05$.
[b] $p < 0.01$.
[c] All data were log transformed to control heterogeneity in variance.

illustrated in Figure 6. No fetal serum sample could be obtained on Day 25 of pregnancy. On Day 40 of pregnancy, the combined effects of high allantoic fluid AP concentrations (~70 μmol/ml per min) and the small fetal blood sample size (~1 ml) resulted in possibly significant contamination of the fetal serum samples with AP from allantoic fluid. Therefore, AP data for these samples were eliminated from the analyses. Fetal serum AP concentrations were not different from Day 30 to Day 70, decreased from Day 70 to Day 80 ($p < 0.05$), and were then not different from Day 80 to Day 90. Fetal serum RBP concentrations increased from Day 30 to Day 40 ($p < 0.01$), decreased from Day 40 to Day 50 ($p < 0.05$), increased again from Day 50 to Day 60 ($p < 0.05$), and were then not different to Day 90. Fetal serum TF increased from Day 30 to Day 40 ($p < 0.01$) and from Day 40 to Day 50 ($p < 0.05$). Concentrations of TF were not different from Day 50 to Day 60; they then decreased from Day 60 to Day 70 ($p < 0.01$) and from Day 70 to Day 80 ($p < 0.05$). The TF concentrations were then not different from Day 80 to Day 90. Correlation analyses indicated that both RBP and TF were significantly correlated with AP in most of the fluids examined (Table 1). Correlations with AP were greatest for TF in allantoic fluid and fetal serum.

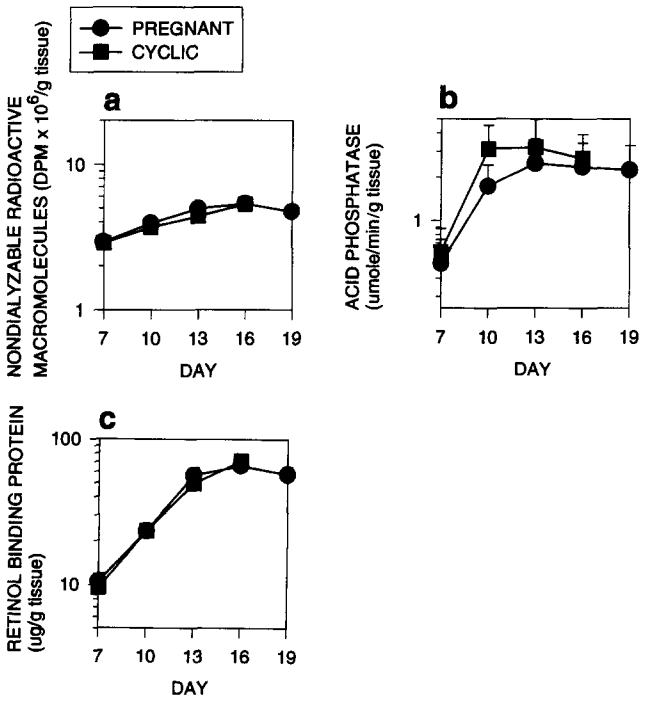

FIG. 3. Least-squares means (± SEM) for NRM (**a**), AP (**b**), and RBP (**c**) secreted in culture by endometrial tissue from cyclic and early pregnant gilts are illustrated. All three increased ($p < 0.01$) from Day 7 to Day 13 and were correlated (see Table 1). Note the log scales.

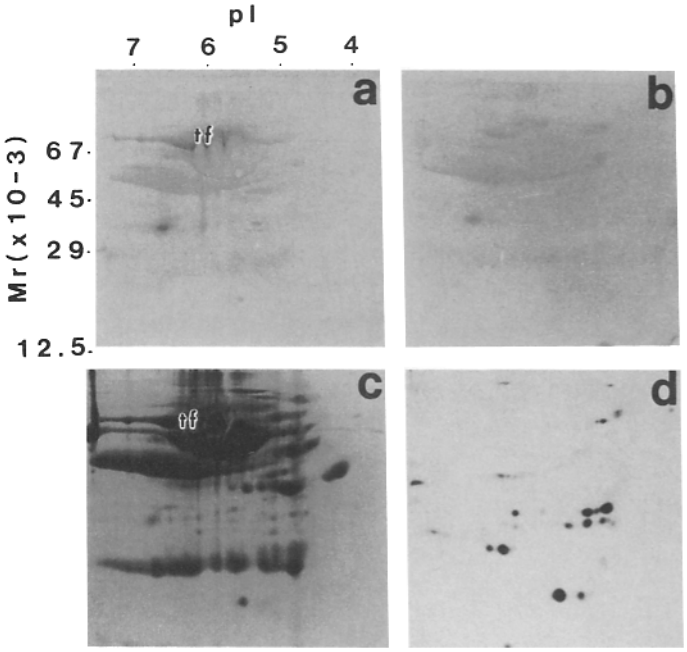

FIG. 4. Representative immunoblots using anti-human TF (**a**) or normal goat serum (**b**), a Coomassie-stained gel (**c**), and a fluorograph (**d**) of 2D-PAGE gels of proteins secreted in culture by endometrium from Day 16 pregnant gilts. Transferrin is the large immunostained spot (tf) at approximately 80 000 M_r, pI 6; no corresponding radioactive protein was detected on the fluorograph.

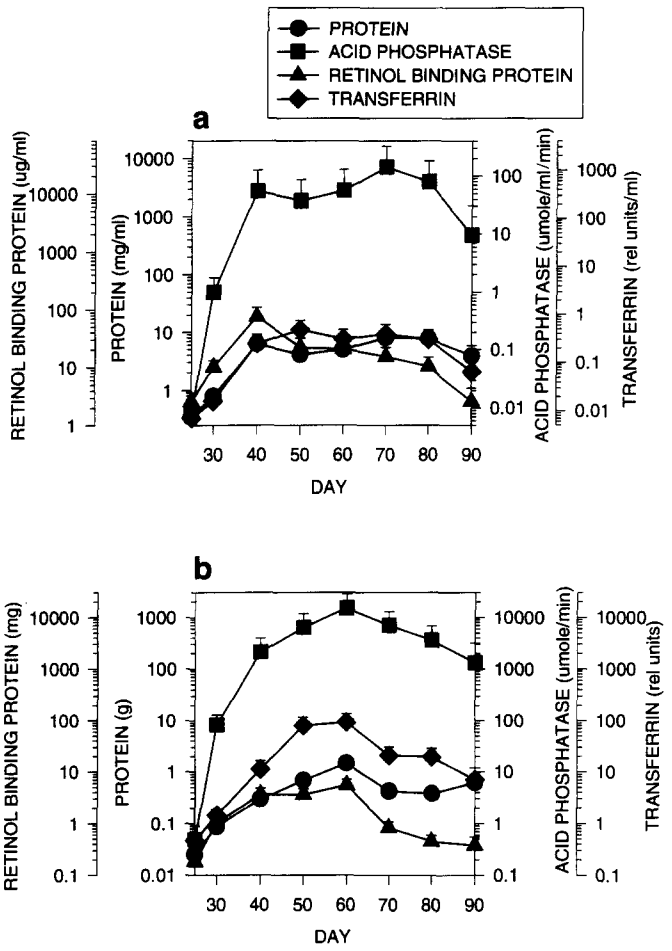

FIG. 5. Least-squares means (± SEM) for protein, AP, RBP, and TF concentrations (**a**) and content (**b**) in allantoic fluid throughout pregnancy are illustrated. Content and concentration of all four increased ($p < 0.01$) between Day 25 and Day 40 of pregnancy. Note the log scales.

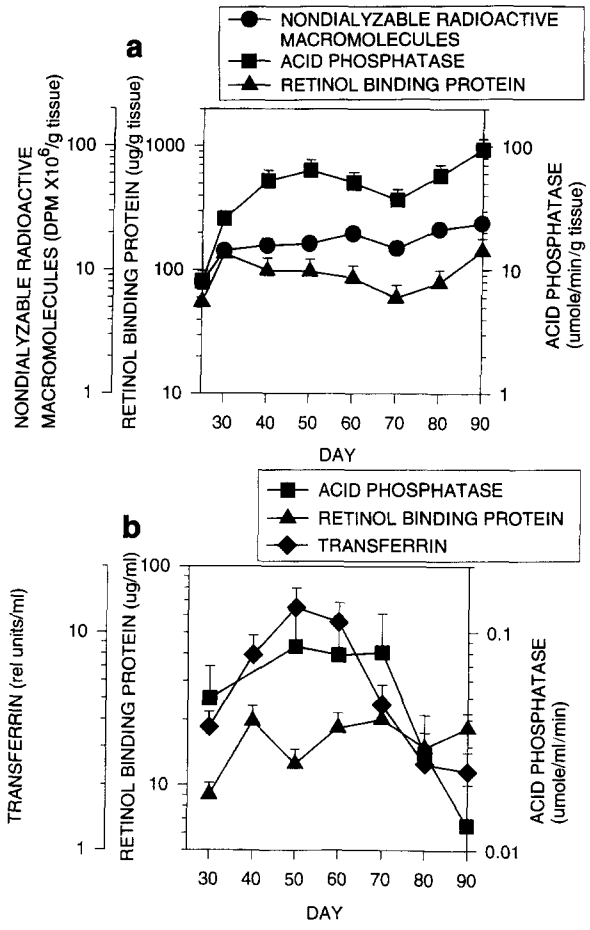

FIG. 6. Least-squares means (± SEM) for endometrial secretion (**a**) of NRMs, AP, and RBP, and fetal serum concentrations (**b**) of AP, RBP, and TF are illustrated. Endometrial secretion of all three increased ($p < 0.01$) from Day 25 to Day 30; AP increased further ($p < 0.01$) from Day 30 to Day 40. Fetal serum RBP increased from Day 30 to Day 40; TF increased ($p < 0.01$) from Day 30 to Day 50. Note the log scales.

DISCUSSION

Our results indicate that RBP and TF concentrations and content in various intrauterine/fetal compartments are associated with concentrations and content of UF, measured as AP. The association is particularly strong in uterine flushings during the estrous cycle and early pregnancy. This association between RBP, TF, and AP is consistent with our hypothesis that RBP and TF may serve to protect uterine and conceptus tissues from the lipid peroxidation activity of UF [7]. Peak concentrations of these three proteins in uterine flushings did not differ between cyclic and pregnant gilts, suggesting that other factors besides conceptus estrogen control their secretion. Although changes in TF content in uterine flushings were detected, no evidence for secretion of this protein by endometrial tissue in culture was obtained, suggesting that this protein may originate from serum. Finally, endometrial secretion of AP after Day 25 of pregnancy increased earlier and persisted later than previously reported [6].

Positive correlations ($p < 0.01$) of greater than 0.9 were obtained between AP, RBP, and TF in uterine flushings during early pregnancy. The correlation between RBP and AP persisted after the data were corrected using protein concentrations as a covariate. Lesser, but still statistically significant, positive correlations were obtained between these proteins in allantoic fluid. In fetal serum, only TF was correlated with AP. Previous results indicated that TF was a more effective inhibitor of lipid peroxidation caused by UF than was RBP [7]. This effectiveness, combined with the close association between TF and AP in all the fluids studied, suggests that TF could be the primary defense against UF-induced lipid peroxidation that may occur as a consequence of the transport of iron via UF secretion. Retinol-binding protein may provide secondary protection during periods when TF concentrations are inadequate to fully protect reproductive tissues. Such periods may occur around Day 13, when intrauterine AP is maximal but TF is not, and around Day 30, when fetal serum and allantoic fluid TF are not yet maximal but serum AP is high and endometrial secretion of AP is approaching maximum. This concept is consistent with the close association between AP and RBP during early pregnancy and with the fact that RBP secretion peaks at Day 30 and then declines until Day 70. The temporal coincidence between these two potentially vulnerable periods and periods of known conceptus loss [16, 23–26] suggests the possibility that failure to adequately protect the conceptus during these periods may be partially responsible for the losses that occur. However, it is also possible that these and other protective mechanisms are more than adequate to protect tissues from damage; the association between the occurrence of lipid peroxidation in conceptus tissue and embryonic or fetal losses has not been

investigated. Further work will be required to determine the validity of this hypothesis.

Results indicate that secretion of RBP by endometrial tissue in culture was highly correlated ($r = 0.95$) with intrauterine concentrations of RBP. In contrast, secretion of AP by endometrium was less, but still substantially, correlated ($r = 0.65$) with intrauterine AP. Several explanations for the lower correlation for AP are possible. Retinol-binding protein and AP may differ in the extent to which they are degraded in the uterine lumen. Thus, AP may be slow to turn over in the intrauterine environment, and therefore small changes in secretion rate may cause large changes in the intrauterine environment. Alternatively, secretion of RBP and AP may differ in the availability of substrate for their respective synthesis. Because MEM contains neither retinol nor iron, endometrial synthesis of each protein may be dependent on intracellular availability of each respective ligand. In any case, intrauterine concentrations reflect more accurately the intrauterine environment to which the conceptus is exposed; thus the relationship within the intrauterine environment takes precedence.

It has been reported that conceptus estrogen stimulates endometrial protein, AP, and RBP secretion during early pregnancy [10, 27–29]. No difference between pregnant and nonpregnant gilts was detected for intrauterine content of protein, AP, RBP, or TF on Days 13 and 16. The lack of an effect of pregnancy on AP agrees with a previous report [5]. The current results indicate that if conceptus estrogen does influence secretion of these proteins, it must do so for only a limited period (i.e., between Day 10 and 13). Clearly, in cyclic gilts, the large increase in secretion of these proteins must be controlled by something other than conceptus estrogen. The timing of this change in protein secretion coincides with decreased progesterone receptor content in epithelial cells of the endometrium [30], a phenomenon that occurs despite the fact that progesterone is still required to maintain pregnancy and secretion of both UF [28, 31, 32] and RBP [3, 10] by the endometrium. It is tempting to speculate that whatever mediates the effect of progesterone within the endometrium after this time may control protein secretion.

Results indicate that intrauterine content of TF increases between Days 7 and 16 of pregnancy and that this increase occurs in both cyclic and pregnant gilts. Thus, the TF present is unlikely to originate from the conceptus. Attempts to demonstrate secretion of TF by Day 16 endometrium in culture suggested that TF is not a detectable endometrial secretory product and that its most likely origin is maternal serum. The TF could enter the intrauterine environment from serum by passive or active transport mechanisms. Endometrial cells may take up holoTF (e.g., TF plus iron) via the TF receptor from serum on the basal side, use the iron to make UF, and release a portion of the apoTF on the apical side (i.e., into the uterine lumen). How intrauterine TF concentrations are controlled will likely depend on the mechanism of transport.

Results suggest that secretion of AP by endometrium in culture is stable from Day 10 to 19 and then increases to Day 40. Both the increase and the peak in secretion were earlier than reported by Basha et al. [6]. Furthermore, although UF secretion decreased from Day 60 to 70, it increased again between Day 80 and Day 90, a result that also differs from those of Basha et al. [6]. The reason for the difference between this and previous reports is not apparent. However, the current results are consistent with measurements of endometrial UF mRNA concentrations [33], which do not decrease during late pregnancy. Despite the observation that endometrial secretion of AP increased from Day 70 to 90, AP in the plasma and in allantoic fluid decreased during this period. This may reflect an increase in iron utilization by the fetus during this period as fetal weight increases rapidly and bone marrow blood synthesis becomes increasingly important [34]. The fall in plasma and allantoic fluid AP suggests that the balance between the secretion rate and utilization rate of UF in fetuses after Day 70 of pregnancy may shift toward increased utilization. Neonatal pigs are known to be deficient in iron [35], further suggesting that the balance between iron transport and utilization during late pregnancy may be less than optimal. Further stress on the system, such as that caused by intrauterine crowding, could perturb this balance in a variety of ways, and therefore the effect of intrauterine crowding on iron balance during late pregnancy should be investigated.

Fetal serum TF concentrations also fall between Days 70 and 90 of pregnancy. If the previously mentioned interpretation of decreased AP concentrations during this period of pregnancy is true, the decrease in TF is a curious result. In neonatal pigs, iron deficiency leads to increased serum TF concentrations [35]. One explanation is that iron from a source other than UF may be provided during this period of pregnancy. Another possibility is that control of TF concentrations may differ in the fetus compared to the neonate with regard to iron deficiency. Concentrations of TF may be controlled by developmental factors in the fetus and be unresponsive to iron concentrations. Further information is needed about modes of iron transport during late pregnancy and factors that control TF in serum during fetal life.

Fetal serum AP, TF, and RBP were not correlated with allantoic fluid AP, TF, and RBP, respectively. This lack of correlation may have been observed for several reasons. It may reflect the selectivity of and changes in function of the fetal kidney, which likely controls the flow of proteins into the allantoic sac. It has been reported that RBP is produced by the fetal kidney; thus fetal kidney secretion of this protein may influence allantoic fluid concentrations over and above the influence of serum RBP concentrations. Concentrations of AP are several orders of magnitude greater in allantoic fluid than in serum, suggesting active transport of this protein into the allantoic sac by the fetal kidney. Because UF may potentially damage fetal tissues, this active transport may be a way of storing UF where it cannot easily interact with fetal tissues (i.e., in a large volume of allantoic fluid). It has been reported that the iron from UF in allantoic fluid can be reabsorbed by the placenta after it is transferred to TF. Uteroferrin itself cannot escape the allantoic sac [14]. It is tempting to speculate that this arrangement is also an adaptation to control possible oxidative damage caused by UF. Transferrin concentrations are lower in allantoic fluid than in serum. This could reflect either restricted transport into the allantois or, alternatively, efficient uptake as iron is transferred from UF to TF and the holoTF is transported to the fetus. It is therefore not surprising that serum and allantoic fluid concentrations of each protein are not correlated.

In conclusion, concentrations of AP and TF within the uterus during early pregnancy, and in allantoic fluid and serum during later gestation, are strongly correlated. This is consistent with the hypothesis that TF may play a role in protecting uterine and fetal tissues from the lipid-oxidizing activity of UF. Retinol-binding protein is also correlated with AP concentrations in the intrauterine environment and in allantoic fluid during early pregnancy, suggesting that it

too may serve a protective role against lipid peroxidation during this period. During early pregnancy, concentrations of all three proteins increase in uterine flushings from both cyclic and pregnant gilts, suggesting that the conceptus does not influence the concentrations of these proteins. Intrauterine concentrations of AP increase dramatically between Day 10 and 13. Endometrial secretion of AP increases dramatically between Day 19 and Day 40 of pregnancy. While TF, RBP, and other antioxidant mechanisms may be sufficient to protect reproductive tissues, the fact that these two periods of increased UF secretion are temporally associated with known periods of conceptus loss suggests that protection from UF-induced lipid peroxidation may not be fully adequate during these periods. We are currently investigating this possibility.

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